OPERATION OF THE PURINE NUCLEOTIDE CYCLE IN ANIMAL TISSUES

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I. INTRODUCTION

The purine nucleotide cycle (PNC) is a group of three reactions involved in the reversible deamination of adenosine 5'-monophosphate (AMP). Deamination is catalysed by the enzyme adenylylate deaminase (E.C. 3.5.4.6) and it results in the formation of IMP (reaction 1):

\[ \text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3. \]  

(1)

The regeneration of AMP from IMP occurs in two steps (reactions 2 and 3), which are catalysed by adenylosuccinate synthetase (E.C. 6.3.4.4) and adenylosuccinate lyase (E.C. 4.3.2.2):

\[ \text{aspartate} + \text{GTP} + \text{IMP} \rightarrow \text{adenylosuccinate} + \text{GDP} + \text{P}_i, \]  

(2)

\[ \text{adenylosuccinate} \leftrightarrow \text{AMP} + \text{fumarate}. \]  

(3)

The net reaction of the cycle is therefore

\[ \text{aspartate} + \text{GTP} + \text{H}_2\text{O} \rightarrow \text{fumarate} + \text{GDP} + \text{P}_i + \text{NH}_3. \]  

(4)

A description of the discovery of the cycle will be followed by a discussion of its operation in various tissues of vertebrates.
In 1927, Parnas & Mozolowski described how homogenization of skeletal muscle of vertebrates in 0.9% NaCl results in a rapid (<90 s), anaerobic production of ammonia. Ammoniagenesis is greater in minced white muscle than in red muscle, and it also takes place in intact muscle upon electrical stimulation. Parnas et al. (1927) subsequently measured ammonia levels in blood drawn from the human cubital vein during rest and during muscular exercise. Blood ammonia proved to be raised three- to fourfold by exercise. Ammonia produced during muscular work therefore seems to be lost to the circulation.

Emden et al. (1928) demonstrated that ammonia production in homogenates of frog and rabbit muscle is not increased by addition of exogenous urea, but strongly stimulated upon addition of AMP, suggesting AMP to be the ammoniogenic substrate. Embden & Wassermeyer (1928) showed that the muscle extract converts AMP to IMP, so that only one-fifth of the total purine nitrogen is liberated as ammonia.

Schmidt (1928) examined deamination processes in a homogenate from rabbit muscle. Whereas AMP and adenosine were rapidly broken down, adenine was hardly deaminated. AMP-deaminase and adenosine deaminase were found to be different proteins. A partially purified preparation of AMP-deaminase showed a pH-optimum of about 6. Although AMP was very rapidly converted to IMP, ATP proved to be unsuitable as a substrate.

Parnas (1929a, b) developed techniques for the measurement of small amounts of purine nucleotides. Thus, he was able to determine that the ammonia formed in anaerobically stimulated frog muscle is equivalent to the net amount of adenine nucleotide converted to IMP. In the presence of oxygen, however, ammonia formation was much greater than the decline of adenylates or the increase of the IMP-level. Parnas explained this by the farsighted assumption that AMP can be resynthesized from IMP under aerobic conditions, using an amino donor other than ammonia. He even proposed amino acids as the most likely donors of the amino group.

After the discovery of adenylate deaminase by Schmidt in 1928, it lasted until 1955 before the mechanism of the aerobic reamination was elucidated. Abrams & Bentley (1955) studied the process in a high-speed supernatant of rabbit bone marrow. They demonstrated aspartate to be a good amino donor, whereas an energy-rich phosphate compound seemed to be required. Carter & Cohen (1955) partially purified the enzyme from yeast which catalyses reaction (3). The authors rightly assumed adenylosuccinate to be an intermediate in the mechanism for incorporation of the amino group in adenine nucleotides. Subsequently, Lieberman (1956) purified a protein from Escherichia coli which synthesizes adenylosuccinate according to mechanism 2. He showed the enzyme to be specific for GTP as the energy source and for aspartate as the amino donor.

Reactions 2 and 3 were later observed in vertebrate skeletal muscle (Newton & Perry, 1957, 1960; Davey, 1961) and vertebrate liver (Davey, 1961). With the emergence of more sensitive techniques of detection, they have been shown to be ubiquitous in all organisms (from bacteria to man) and in all tissues which are capable of de novo purine synthesis (Lowenstein, 1972).
Early evidence for 'purine nucleotide cycling'

Although a forward and backward mechanism for the conversion of AMP into IMP has been shown to exist, this does not prove that the enzymes AMP-deaminase, adenylosuccinate synthetase and adenylosuccinase operate as a cycle. In theory, it is quite possible for the deamination and reamination pathways to function independently in the catabolism and anabolism of purine nucleotides. If AMP formed by adenylosuccinate synthetase is rapidly converted to ATP, and IMP formed by AMP-deaminase is immediately dephosphorylated, no 'cycling' will occur. The turnover rate of the 6-amino-group of adenine nucleotides will in that case be equal to the turnover of the ring nitrogens. If on the other hand purine skeletons pass several amination/deamination sequences before being ultimately catabolized, purine nucleotides will serve a catalytic function in the liberation of ammonia from aspartate and the turnover of the 6-NH₂ group will be much greater than turnover of nitrogen in the purine ring.

By feeding rats [¹⁵N]ammonium citrate, Barnes & Schoenheimer (1943) demonstrated that ¹⁵N incorporated in nucleic acids of rat liver is fairly equally distributed between the amino-N and the purine ring nitrogens. Liver AMP-deaminase therefore seems to operate more or less independently of the IMP-reamination pathway and there appears to be little purine nucleotide cycling.

In similar feeding experiments, however, Kalckar & Rittenberg (1947) showed that muscle incorporates the label predominantly into the 6-NH₂-group. After 7-8 hours, the amino group was labelled 50 times more strongly than the nitrogens of the purine ring. In muscle, turnover of the amino group is thus much higher than that of the ring nitrogens, indicating the occurrence of active purine nucleotide cycling. Kalckar & Rittenberg's data were later confirmed by Newton & Perry (1957, 1960).

Evidence for a cyclical operation of muscle AMP-deaminase, adenylosuccinate synthetase and adenylosuccinate lyase has also been presented by Wajzer et al. (1956). These authors mounted frog skeletal muscle fibres in the beam of a UV spectrophotometer. Absorption was monitored at different wavelengths, while the fibre was stimulated. A rapid decrease at 265 nm was accompanied by an increase at 240 nm and a stable absorption at 248 nm. After a single contraction, absorption at 240 and 265 nm returned slowly to the control values. This observation suggests that reversible degradation of AMP to IMP takes place during a single muscle twitch. The deamination and reamination steps seemed to be out of phase, deamination occurring during contraction, whereas reamination is part of the recovery from exercise. Later attempts to measure AMP-IMP conversion during a single muscle twitch radiochemically, however, were unsuccessful (Cain et al., 1963).

Operation of the PNC was finally demonstrated in a gel-filtered, dialysed high-speed supernatant of rat muscle (Lowenstein & Tornheim, 1971; Lowenstein, 1972; Tornheim & Lowenstein, 1972). The supernatant was incubated with IMP, GTP and aspartate in the presence of a GTP-regenerating system (creatine phosphate and creatine kinase). Under these conditions, IMP was aminated. A transient accumulation of adenylosuccinate occurred, but eventually all IMP was converted to adenine nucleotides. Ammonia production was negligible, due to inhibition of adenylate deaminase by GTP. This condition mimicks resting muscle under aerobic conditions.
When 2-deoxyglucose and hexokinase were subsequently added to the extract, adenine nucleotides were rapidly converted to IMP. ATP was used to phosphorylate 2-deoxyglucose in the hexokinase reaction. Since deoxyglucose cannot be used as a substrate for glycolysis, high-energy phosphate became trapped in the form of 2-deoxyglucose 6-phosphate and accumulation of AMP occurred via the myokinase reaction. AMP-deaminase was activated, due to an increased availability of substrate and disappearance of GTP, whereas adenylosuccinate synthetase became rapidly inhibited due to the formation of GDP. This situation simulates tetanic exercise.

By repeated addition of creatine phosphate and 2-deoxyglucose, complete amination and deamination of the purine nucleotides was obtained 4 times, indicating the ability of the PNC to operate as a cycle. Formation of ammonia was shown to be dependent on the presence of aspartate and catalytic amounts of IMP (or ATP, or adenylosuccinate) in the muscle extract (Lowenstein, 1972; Tornheim & Lowenstein, 1972).

(3) Proposed physiological functions of the cycle

In any discussion of the operation of the PNC, we should keep in mind that the cycle consists of two components:

(a) Deamination of AMP generates IMP and $\text{NH}_3$. This part of the cycle has no direct relationship with aerobic metabolism, but it may be particularly important during glycolytic energy production.

(b) Reamination of IMP uses the amino group of aspartate and produces fumarate and AMP. This pathway is closely linked to aerobic energy production.

Thus, the following functions of the PNC may be envisaged:

$Ad$ (a)

(1) Stabilization of the adenylate energy charge (ATP/ADP-ratio, or phosphorylation potential)* by the adenylate deaminase reaction in situations when energy demand surpasses energy production.

This function has been proposed by Lowenstein (1972), Chapman & Atkinson (1973) and Chapman et al. (1976). When liver cells (or the intact liver) are incubated with fructose or 2-deoxyglucose, for example, inorganic phosphate becomes trapped in the form of fructose-1-phosphate or 2-deoxyglucose-6-phosphate. Correct ATP/ADP ratios can no longer be maintained by glycolysis and oxidative phosphorylation, since the concentration of inorganic phosphate has dropped so much that it becomes rate-

* The energy charge of a cell is the extent to which the adenine nucleotide pool is ‘charged’ with high-energy phosphate bonds. It is calculated by the formula

$$\text{energy charge} = \frac{[\text{ATP}]+0.5[\text{ADP}]}{[\text{ATP}]+[\text{ADP}]+[\text{AMP}]}.\$$

The energy charge is equal to 1 when all adenine nucleotide is present in the form of ATP; it is 0 when the pool consists only of AMP. The charge concept was originally formulated by Atkinson (1968). An even more sensitive indicator of the energy status of a cell is its phosphorylation potential:

$$\text{phosphorylation potential} = \frac{[\text{ATP}]}{[\text{ADP}][\text{P}]}.$$

Both the energy charge and the phosphorylation potential are a measure for the ability of the cell to carry out ATP-dependent processes.
The purine nucleotide cycle

limiting. In this case, adenylate deaminase is activated, resulting in a decrease of the size of the adenylate pool. The ATP/ADP ratio is increased, since the myokinase equilibrium (reaction 5) is pulled in the direction of ATP-formation:

\[
2\text{ADP} \leftrightarrow \text{AMP} + \text{ATP} \quad (5)
\]

\[
\text{AMP} + \text{H}_2\text{O} \rightarrow \text{IMP} + \text{NH}_3 \quad (1)
\]

Net reaction: \(2\text{ADP} \rightarrow \text{ATP} + \text{IMP} + \text{NH}_3\) \quad (6)

(2) Control of the rate of glycolysis and glycogenolysis via allosteric modulation of phosphofructokinase.

This function has been proposed by Lowenstein (1972) and Sugden & Newsholme (1975). Ammonium ions stimulate phosphofructokinase (PFK), an enzyme that catalyses one of the rate-limiting steps of glycolysis. The activation is not based on any change in pH (Abrahams & Younathan, 1971). Lowenstein (1972) proposed that ammonia liberated in the adenylate deaminase reaction might have a twofold effect:

(i) Direct activation of PFK.

(ii) Indirect activation of PFK by buffering of hydrogen ions (produced during lactate formation). A rise in pH makes PFK much less susceptible to inhibition by its allosteric effector ATP.

Ad (b)

(3) Control of the total level of citric acid cycle intermediates (see Fig. 1). Operation of the PNC will increase the total level of TCA cycle intermediates because of the conversion of aspartate to fumarate. Such an expansion of the pool of citric acid cycle intermediates will lead to an activation of aerobic energy production, since four-carbon 'sparker' molecules are provided. This function was proposed by Lowenstein (1972) and it may be particularly important in tissues which lack alternative anaplerotic mechanisms.

(4) Liberation of ammonia from amino acids. When the net reaction of the cycle (4) is coupled to the fumarase (7), malate dehydrogenase (8) and glutamate-oxaloacetate transaminase (9) reactions, we obtain the following scheme:

\[
\text{aspartate} + \text{GTP} + \text{H}_2\text{O} \rightarrow \text{fumarate} + \text{GDP} + \text{P}_1 + \text{NH}_3 \quad (4)
\]

\[
\text{fumarate} + \text{H}_2\text{O} \leftrightarrow \text{malate} \quad (7)
\]

\[
\text{malate} + \text{NAD}^+ \rightarrow \text{oxaloacetate} + \text{NADH} + \text{H}^+ \quad (8)
\]

\[
\text{glutamate} + \text{oxaloacetate} \leftrightarrow \text{alpha-ketoglutarate} + \text{aspartate} \quad (9)
\]

overall: \(\text{glutamate} + \text{NAD}^+ + \text{GTP} + 2\text{H}_2\text{O} \rightarrow \text{alpha-ketoglutarate} + \text{NADH} + \text{GDP} + \text{P}_1 + \text{NH}_4^+ \quad (10)
\]

Reaction (10) is similar to the oxidation of glutamate by glutamate dehydrogenase (11), the only difference being the hydrolysis of the high-energy phosphate bond of GTP:

\[
\text{glutamate} + \text{NAD}^+ \rightarrow \text{alpha-ketoglutarate} + \text{NADH} + \text{NH}_4^+. \quad (10)
\]
Fig. 1. Anaplerotic role of the purine nucleotide cycle. Operation of the cycle results in the formation of fumarate in the cytosolic compartment. Fumarate is converted to malate by fumarase and transported into the mitochondria. The pool of four-carbon 'sparker' molecules (malate, oxaloacetate) is thus increased, resulting in a more rapid oxidation of acetyl-CoA by the Krebs cycle.

The free energy of reaction (10) is about \(-1.0\) kcal/mol, that of reaction (11) approximately +6.5 kcal/mol (Lowenstein, 1972). The PNC is therefore poised towards oxidative deamination of glutamate, whereas the glutamate dehydrogenase reaction is poised towards reductive amination of alpha-ketoglutarate. Reaction (11) can only proceed in the indicated direction when the end products, alpha-ketoglutarate and NADH, are rapidly catabolized.

As evidence to support this function, Lowenstein (1972) presented the following data:

(i) Glutamate dehydrogenase and adenylate deaminase show reciprocal tissue distributions, a high activity of the former being accompanied by low activity of the latter and vice versa. This suggests glutamate dehydrogenase and the PNC to be alternative pathways for amino acid deamination.

(ii) Mitochondria from a variety of tissues do not normally liberate ammonia from glutamate. Glutamate catabolism proceeds in these cases via a transamination reaction involving oxaloacetate with the resultant production of aspartate (see Borst, 1962; de Haan et al., 1967; Krebs & Bellamy, 1960; Van Waarde & De Wilde-van Berge Henegouwen, 1982). Ammonia production by these tissues therefore seems to require extramitochondrial deamination of aspartate via the PNC.

(5) Control of the relative amounts of adenine and guanine nucleotides in the cell (see Fig. 2). Adenylates can be converted into guanylates by the action of AMP-deaminase, IMP dehydrogenase and GMP synthetase. Guanylate/adenylate conversion involves GMP-reductase, adenylosuccinate synthetase and adenylosuccinate lyase. GMP-
The purine nucleotide cycle

Fig. 2. Relationship between the purine nucleotide cycle and the interconversion of adenine and guanine nucleotides. The numbered enzymes are: 1, Adenylosuccinate synthetase (E.C. 6.3.4.4); 2, Adenylosuccinate lyase (E.C. 4.3.2.2); 3, AMP deaminase (E.C. 3.5.4.6); 4, IMP dehydrogenase (E.C. 1.2.1.14); 5, GMP synthetase (E.C. 6.3.5.2); 6, GMP reductase (E.C. 1.6.6.8).

Synthetase requires ATP, whereas adenylosuccinate synthetase uses GTP. AMP-deaminase is inhibited by GTP and GMP-reductase activity is depressed by ATP (Henderson & Paterson, 1973). These regulatory properties help the cell to maintain the proper balance between the levels of adenine and guanine nucleotides.

II. LIVER

When D-fructose (10 mM) is infused in rats, striking metabolic changes occur in the liver. Within 10 minutes fructose-1-phosphate accumulates to a level of 8.7 μmol/g wet weight. The ATP-content decreases to 23% of the control value, whereas IMP rises sevenfold to a maximum of 1.1 μmol/g (Woods et al., 1970).

Fructose-1-phosphate is formed by the activity of fructokinase (E.C. 2.7.1.3):

\[ \text{fructose} + \text{ATP} \rightarrow \text{fructose-1-phosphate} + \text{ADP}. \]  (12)

The high rate of this reaction causes a rapid decrease in the concentrations of ATP and inorganic phosphate. Due to the decrease of the value of the adenylate energy charge and the disappearance of inorganic phosphate, AMP-deaminase becomes activated and AMP is deaminated to IMP. Fructose-1-phosphate is under normal conditions removed by the action of aldolase (E.C. 4.1.2.13), which has an activity equal to that of fructokinase:

\[ \text{fructose-1-phosphate} \leftrightarrow \text{dihydroxyacetonephosphate} + \text{D-glyceraldehyde}. \]  (13)

The accumulation of fructose-1-phosphate which occurs in the presence of exogenous fructose therefore needs an explanation. According to Woods et al. (1970), aldolase is inhibited by IMP and fructose-1-phosphate accumulation thus becomes a prolonged phenomenon.
Chapman & Atkinson (1973) examined the kinetic properties of rat liver AMP-deaminase, which are responsible for the decline of the adenylate pool and the increase of IMP in the presence of fructose. A partially purified preparation of the enzyme proved to be activated by ATP under all conditions examined. The effect is largely on the affinity of the enzyme for its substrate, $K_m$ values for AMP being lowered from 11.1 to 0.3 mM in the presence of ATP. Liver AMP-deaminase is also activated by ADP, but to a lesser degree. The authors evaluated the response of the enzyme to variations in the energy charge at a constant size of the adenylate pool. In the physiological range of energy charge ($0.7-0.9$), the rate of deamination increases sharply with decreasing charge. When the charge drops below 0.6, however, the activity of the enzyme decreases again. Chapman & Atkinson (1973) therefore suggested that liver AMP-deaminase serves to protect the organ against transient decreases of the energy charge during situations in which there is an imbalance between ATP-consumption and ATP-production. The decrease of enzyme activity at levels of charge below 0.6 was explained as a self-limiting response to prevent excessive depletion of the adenine nucleotide pool. Protection of the cell against short-term decrease in energy charge is at the expense of the adenylate pool size, but the dependence of AMP-deaminase activity on the adenylate pool size provides a built-in limit on how far the pool can be reduced. Because of the limited amount of adenine nucleotides present in the cell, stabilization of the energy charge by the adenylate deaminase reaction cannot be a long-term effect; it can protect only against sharp drops in charge during the transient period while a new steady-state is established (in which ATP-formation and ATP-consumption are once more equal).

Van den Berghe et al. (1977) made a detailed study of the metabolic changes occurring in mouse liver after fructose loading. They showed that fructose-1-phosphate accumulates during the first minute after the onset of fructose infusion, whereas IMP-accumulation occurs only during the second minute. Therefore, IMP-inhibition of aldolase is not the mechanism responsible for fructose-1-phosphate accumulation. The apparent blockade of aldolase seems to be caused by the fact that the following steps in the glycolytic chain cannot keep pace with the fructokinase and aldolase reactions. On the basis of metabolite measurements, Van den Berghe et al. (1977) proposed that the disappearance of GTP is one of the signals leading to deinhibition of AMP-deaminase and IMP-accumulation. An additional mechanism might be the drop of the inorganic phosphate concentration (Oberhansli et al., 1987).

When liver AMP-deaminase is inhibited by a high dose of coformycin (50 μM), the fructose-induced breakdown of ATP is unaffected, but the depletion of the adenine nucleotide pool proceeds much more slowly (Van den Berghe et al., 1980). There is a strong increase in the level of AMP, whereas IMP does not accumulate at all. 5'-Nucleotidase seems to be inactive, unless the AMP-concentration rises to unphysiological values. As a consequence, the drop of the liver adenylate energy charge after fructose administration is much greater in coformycin-treated rats than in control rats (Van den Berghe et al., 1980, see Table 1).

A similar degradation of adenylates and accumulation of IMP due to activation of AMP-deaminase is observed in liver cells during environmental anoxia (Vincent et al., 1982).

The role of glutamate dehydrogenase and the PNC in urea formation from amino...
Table 1. Changes in metabolite levels (μmoles/g wet weight) of rat hepatocytes after treatment with fructose

(Data of Van den Berghe et al. (1980).)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control rats</th>
<th>Coformycin-treated rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>2.0</td>
<td>2.3*</td>
</tr>
<tr>
<td>ADP</td>
<td>0.8</td>
<td>0.7*</td>
</tr>
<tr>
<td>AMP</td>
<td>0.1</td>
<td>0.1*</td>
</tr>
<tr>
<td>TAN</td>
<td>2.9</td>
<td>3.1*</td>
</tr>
<tr>
<td>AEC</td>
<td>0.83</td>
<td>0.85</td>
</tr>
</tbody>
</table>

No fructose Fructose 2 m  No fructose Fructose 2 m

TAN = Total level of adenine nucleotides; AEC = Adenylate energy charge.
* Significant difference between 'No fructose' and fructose-treated groups.
† Significant difference between control and coformycin-treated rats.

acids has for some time been a subject of discussion. In mammals, urea is the end product of protein catabolism and is synthesized from aspartate and ammonia by carbamyl phosphate synthetase and the urea cycle. It has been generally believed that the free ammonia which is necessary for urea formation arises from the action of glutamate dehydrogenase in the mitochondrial matrix (Lehninger, 1970).

This assumption was questioned during the mid-seventies in several publications. Experiments using rat liver mitochondria suggested that the in vivo activity of glutamate dehydrogenase is too low to account for the observed rates of urea synthesis, due to a slow entry of glutamate in the mitochondrial matrix (see McGivan et al., 1974; McGivan & Chappell, 1975). The amino acid leucine accelerates glutamate dehydrogenase in sonicated mitochondria, but it inhibits urea synthesis in intact hepatocytes. On the basis of these observations, McGivan and co-workers suggested that glutamate dehydrogenase does not play any role in liver amino acid deamination. The enzyme would function primarily in the direction of glutamate formation as a mechanism for nitrogen storage, whereas the ammonia necessary for urea synthesis would originate in the cytoplasmic PNC (McGivan & Chappell, 1975; Moss & McGivan, 1975; Mendes-Maurad et al., 1975; Sies et al., 1975).

However, the argumentation which was presented in support of a function of the PNC in urea synthesis was subsequently invalidated by observations of Rognstad (1977) and Krebs et al. (1978). Rognstad (1977) demonstrated that leucine inhibition of urea synthesis in intact hepatocytes can be overcome by administration of ornithine, which suggests that the main site of leucine action is at an enzyme from the urea cycle, ornithine transcarbamylase, rather than at glutamate dehydrogenase. He also showed that the formation of glucose and lactate from asparagine in intact hepatocytes can be strongly suppressed by addition of amino-oxyacetate, an inhibitor of transamination, whereas hadacidin, an inhibitor of the purine nucleotide cycle, has no effect on urea synthesis. Krebs et al. (1978) demonstrated that the initial rate of incorporation of [15N]alanine into the 6-amino group of adenine nucleotides in rat hepatocytes is about one-eighteenth of the rate of incorporation into urea. These results suggest that,
contrary to the opinion of the previous authors, the PNC is not a major pathway for ammonia production in rat liver. In the intact organ, glutamate dehydrogenase seems to be perfectly able to sustain normal rates of amino acid deamination.

A similar conclusion is valid for the liver of ammoniotelic fish. In goldfish hepatopancreas, activity of glutamate dehydrogenase (in the direction of oxidative deamination) is 100-fold greater than that of adenylosuccinate synthetase, the rate-limiting step of the PNC (Van Waarde, 1981). Endogenous ammonia production of goldfish hepatocytes (corrected for net adenylate degradation) is unaffected by the transaminase inhibitor amino-oxy-acetate or the PNC-inhibitor hadacidine (Van Waarde & Kesbeke, 1981b). Ammonia production from endogenous leucine, however, is completely blocked by amino-oxy-acetate and only slightly inhibited by hadacidine (Van Waarde & Kesbeke, 1981b). These data suggest that amides (asparagine, glutamine) and glutamate are the major endogenous ammoniogenic substrates (see also French et al. 1981; Campbell et al., 1983), whereas glutamate dehydrogenase is the enzyme involved in amino acid deamination (Van Waarde & Kesbeke, 1981b).

Using $^{15}$N-labelled amino acids, Casey et al. (1983) measured the rate of [$^{15}$N]-incorporation into ammonia and the 6-amino group of the purine nucleotides by liver cells of the channel catfish. The rate of appearance of the label in ammonia proved to be much higher than in the purine amino group. Therefore, the PNC is not a significant mechanism for amino acid deamination in fish hepatocytes. The evidence available for livers of catfish, goldfish and other fish species indicates that the major route for amino acid catabolism is the transdeamination pathway.

III. KIDNEY

A very important function of the kidney is the production of ammonia for maintenance of the acid-base balance. In response to acid loads, excess protons are combined with ammonia and excreted as ammonium ions. Production and excretion of ammonia are therefore greatly elevated in metabolic acidosis.

It has been well established that most of the ammonia formed in kidney originates from the amide and amino nitrogens of glutamine. The deamidation reaction which converts glutamine to glutamate is known to be catalysed by phosphate-dependent glutaminase (E.C. 3.5.1.2), but the question, which route is followed for deamination of glutamate, has been hotly debated in recent literature. Major candidates are the glutamate dehydrogenase reaction (E.C. 1.4.1.2) and the PNC. The presence of the latter pathway in rat kidney has been demonstrated by Bogusky et al. (1976). During ischaemia, IMP accumulates in kidney of mouse (Warnick & Lazarus, 1981) and rats (Gerlach et al., 1963). Turnover of the 6-amino group of adenine nucleotides in kidney is substantial, approximately 3-4 times greater than in liver.

The effect of acid feeding (120 h) and recovery (48 h) on the metabolism of rat kidney has been examined by Bogusky et al. (1981). Acidosis causes an 8-fold increase of the level of IMP, whereas adenylosuccinate decreases to 25% of the control value. After cessation of acid feeding, normal IMP-levels are rapidly restored, but adenylosuccinate declines further and drops below the limit of detection. Kidney $\alpha$-ketoglutarate content decreases q-fold during acidosis and returns to normal after termination of the acid intake.

The activities of adenylosuccinate synthetase, adenylosuccinate lyase, glutaminase
and glutamate dehydrogenase are 2-2.5 fold increased during acidosis, whereas AMP-deaminase is unchanged. Activity of adenylosuccinate synthetase, the rate-limiting step of the PNC, parallels the rise and fall in ammonia excretion, but glutaminase and glutamate dehydrogenase remain elevated during at least 48 h of the recovery period, when ammonia excretion has returned to the control rate.

The authors concluded that changes in the activity of the PNC correlate with changes in ammonia excretion to a more parallel degree than do the activities of glutaminase or glutamate dehydrogenase. They proposed that the PNC may play a regulatory role in renal ammoniogenesis. It is evident, however, that the activity of adenylosuccinate synthetase is quite minor compared to the high levels of glutaminase and glutamate dehydrogenase observed under the same conditions.

A significant role of the PNC has also been proposed on the basis of experiments using enzyme inhibitors (Bogusky et al., 1983). Isolated kidney tubules were incubated with 2-amino-4-methoxy-trans-but-3-enoic acid (MVG), an inhibitor of transamination. The drug has no effect on the activity of the enzymes of the PNC or on glutamate dehydrogenase. In the presence of MVG, transamination is blocked so that ammonia from exogenous aspartate can only be produced via the PNC. Ammonia production from exogenous glutamate, however, can proceed only via the glutamate dehydrogenase reaction. A comparison of the rates of ammonia production from aspartate and glutamate under these conditions will therefore give information about the relative capacity of the PNC and the glutamate dehydrogenase reaction to produce ammonia in the intact kidney cell. The results showed that ammonia can be formed by glutamate dehydrogenase and the PNC at significant and approximately equal rates. It should be noted, however, that the experiments cannot be considered proof of ammoniagenesis by the PNC in the normal situation. Although aspartate metabolism must proceed via the PNC in the presence of MVG, in its absence the pathway may be different. An inhibitor might merely divert the flow from a major to a normally minor pathway.

Bogusky & Aoki (1983) studied ammonia production in the isolated perfused rat kidney, using [15N]glutamate as a substrate. Before isolation of the kidney, rats were treated with the glutamine synthetase inhibitor methionine sulfoximine to simplify calculation of the nitrogen balance.

With 2 mM [15N]glutamate and 5 mM glucose as exogenous substrates, [15N]-incorporation into the 6-amino group of adenine nucleotides seemed to be earlier and to be much higher than the appearance of 15N into free ammonia. For this reason, the authors assumed that glutamate deaminated by the PNC was the primary source of ammonia.

When the tricarboxylic acid cycle was inhibited at the level of aconitase with 0.1 mM fluorocitrate, ammonia production was increased fourfold above the control level. From simultaneous measurements of the levels of endogenous amino acids and purine nucleotides, Bogusky & Aoki (1983) concluded that also in this case, glutamate was the main ammoniogenic substrate.

During the first 5 minutes after addition of fluorocitrate, both the turnover rate of the 6-amino group of adenine nucleotides and kidney IMP-content showed a more than threefold increase, which was accompanied by a rapid fall in the tissue levels of glutamate and aspartate. The authors therefore proposed that ammoniagenesis in the
presence of fluorocitrate occurs initially via the PNC. After 5 minutes, however, the ATP-level showed a 50\% decline and IMP-content and the incorporation of $^{15}$N into the purine amino group decreased rapidly. The PNC seemed to be inhibited and ammonia formation proceeded now via the glutamate dehydrogenase reaction in amounts stoichiometric with uptake of glutamate from the perfusate.

Ammonia could also be formed from exogenous aspartate at a rate of 1.0 \(\mu\)mol/min.g. After addition of fluorocitrate, ammonia production and renal uptake of aspartate showed a parallel decline, but tissue levels and uptake rates of glutamine and glutamate were unaffected. Since fluorocitrate inhibits the PNC, whereas glutamate dehydrogenase is stimulated, Bogusky & Aoki (1983) concluded that all ammonia produced from aspartate is formed via the PNC and that the PNC can make ammonia from aspartate at a rate of 1.0 \(\mu\)mol/min.g, which is equal to the rate of ammonia formation by glutamate dehydrogenase.

It should be noted, however, that the conditions which were used in their study were unphysiological, since methionine sulfoximine can be expected to have a variety of effects on kidney nitrogen metabolism. It is also possible to challenge the evidence they presented in support of a major function of the PNC in glutamate deamination. They compared labelling of free ammonia and the purine amino group by examination of the atom \% excess $^{15}$N in both types of compounds. No correction was made for the pool sizes of the metabolites. Since very high levels of unlabelled ammonia were present at the beginning of the experiments and since the total amount of adenine nucleotides in the kidney is relatively small, pool sizes have to be taken into consideration. When the absolute amounts of $^{15}$N present in ammonia and adenine nucleotides in Bogusky & Aoki's experiments are calculated from the atom \% excess $^{15}$N and the respective pool sizes, it can be shown that in reality, incorporation of label in ammonia was much higher (and not lower) than in the purine amino group (see the discussion in Tornheim et al., 1986). The conclusion that the PNC is the major pathway for kidney glutamate deamination has therefore not been substantiated. Perfusion of a kidney with high levels of aspartate creates an artificial situation, since in the intact animal plasma levels of aspartate are very low.

Strzelecki et al. (1983) conducted studies with slices of rat kidney cortex, using $[^{15}N]$glutamine as substrate. $^{15}$N incorporation in the amino group of adenine nucleotides proved to be negligible under these conditions. Addition of inorganic phosphate (a strong inhibitor of AMP-deaminase) had no effect on ammonia formation from glutamine. When intracellular P$_i$ was lowered by administration of fructose (a condition in which AMP-deaminase can be expected to be stimulated), ammonia production rates were also unaltered. Strzelecki et al. (1983) therefore suggested that the contribution of the PNC to ammonia formation from glutamine is insignificant. It is possible, however, to deny the validity of that conclusion because of the unfavourable energetic condition of the slices. Although no net adenylate degradation occurred during the course of the experiment, the adenylate energy charge had the very low value of 0.59 (Table 2 in their paper). Under such conditions, which differ vastly from the in vivo situation, the PNC can be expected to be inhibited at the level of adenylosuccinate synthetase.

Nissim et al. (1985, 1986) and Tornheim et al. (1986) studied the metabolism of $[^{15}N]$glutamine, $[^{15}N]$glutamate and $[^{16}N]$aspartate in isolated kidney tubules. Tubules
are a much better preparation for the study of kidney metabolism than slices, since they maintain levels of high-energy phosphates similar to those of the intact organ and are capable of active ion transport. The intactness of the tubule preparation can be inferred from the fact that the adenylate energy charge was maintained at a level of 0.83–0.87 during all experimental treatments (Nissim et al., 1986, calculated from their table 11). Nissim et al. (1985) made a very detailed study of $[^{15}N]$incorporation in many metabolites (not only purine nucleotides and ammonia, but also alanine and aspartate), using the sensitive technique of gas chromatography–mass spectrometry (GC–MS). Tubules from chronically acidic rats were compared to tubules isolated from normal rats.

Experiments in which the amide group of glutamine was labelled showed that in control animals approximately 90% of ammonia nitrogen arises by deamidation versus 60% in tubules from acidic rats. Experiments in which the amino group of glutamine, or the amino group of glutamate were labelled, indicated that in acidosis approximately 30% of ammonia nitrogen is derived from the amino group by the activity of glutamate dehydrogenase. Control rats did not form $^{15}$NH$_3$ from [2-$^{15}$N]glutamine. Transamination rates and transfer of $^{15}$N to the amino group of purine nucleotides were much higher in tubules from normal rats than in a preparation from chronically acidic rats. In tubules from acidic rats, the total amount of $^{15}$N and the rate of appearance of $^{15}$N in free ammonia were much (i.e. 80–100 fold) higher than in the purine amino group. Nissim et al. (1985) interpreted these data as showing that glutamate dehydrogenase rather than the PNC is responsible for the increased ammoniagenesis which occurs in acidosis. Since no precursor–product relationship was observed between the 6-amino group of purine nucleotides and free ammonia, the contribution of the PNC to ammoniagenesis appears to be relatively unimportant in chronically acidic rats. In tubules from normal rats incubated with [2-$^{15}$N]glutamine, however, the isotopic enrichment of the 6-amino group of purine nucleotides was higher and faster than that of free ammonia. In normal acid-base status, the turnover of the adenine nucleotides seems therefore to be able to account for the ammonia formed from glutamate (Nissim et al., 1985).

In order to evaluate further the operation of the PNC, Nissim et al. (1986) incubated tubules with $[^{15}N]$aspartate and 5-amino-4-imidazolecarboxamide riboside (AICARiboside), a precursor of IMP. In tubules isolated from control rats, low levels of AICARiboside (1 mM) significantly stimulated production of labelled ammonia and incorporation of label in the amino group of purine nucleotides. The stimulation of ammonia production from aspartate probably took place via the PNC rather than the glutamate dehydrogenase reaction, since the rate of $[^{15}N]$glutamate formation from $[^{15}N]$aspartate was not changed by AICARiboside. However, a high dose (5 mM) of the drug inhibited ammonia production and reduced tubule purine nucleotide content. This biphasic influence of the riboside can be explained on the basis of its well-documented effects on purine metabolism (see, e.g. Sabina et al., 1982; Swain et al., 1984). Low doses of AICARiboside stimulate the PNC by increasing the availability of IMP. At high doses, however, intracellular AICARibotide accumulates and adenylosuccinate lyase, one of the enzymes of the PNC, is inhibited. In tubules from acidic rats, isotopic enrichment in ammonia was higher and faster than in the 6-amino group of the adenine nucleotides, i.e. no precursor-product relationship was observed.
between the nucleotides and ammonia. In tubules from control rats, however, enrichment in the 6-amino group of adenine nucleotides exceeded that in ammonia. The mentioned relationships were observed whether or not AICArriboside was included in the incubation medium. The experiments of Nissim et al. (1986) therefore suggest that during normal acid-base balance, the PNC plays a small role in ammoniagenesis. Even in the acidic state some ammonia seems to be formed by the PNC.

Somewhat different conclusions were reached by Tornheim et al. (1986), who incubated renal cortical tubules (isolated from acidotic rats) with [2-\(^{15}\)N]glutamine, [\(^{15}\)N]glutamate and [\(^{15}\)N]aspartate. They found no (<10\%) incorporation of label into adenine nucleotides, even after prolonged incubation (1 h), although the energy charge of the tubules was normal. From the measured concentrations of adenine nucleotides and ammonia, and the labelling of ammonia, Tornheim et al. (1986) calculated that the flux through the PNC accounted for less than 1% of the deamination of alpha-amino groups from all three substrates. Thus, the PNC was considered insignificant for ammoniagenesis in tubules from acidotic rats. In tubules from normal rats there was a greater incorporation of label into adenine nucleotides and amino acids, accompanied by a reduced release of ammonia. Therefore, the PNC seems to play some role in basal ammoniagenesis.

In summary, it can be said that the PNC is not the major deaminating pathway in vertebrate kidney. Some cycling does occur, however, especially in the situation of normal acid-base balance.

IV. PLATELETS

AMP-deaminase activity of human platelets can be almost totally blocked by a 6 h incubation in the presence of 200 \(\mu M\) coformycin (Ashby et al. 1983; Ashby & Holmsen, 1983). Addition of thrombin to a platelet suspension induces shape change, secretion and aggregation of the cells. Secretion is accompanied by purine breakdown via AMP-deaminase and a burst of glycolysis ending in lactate production. Blocking of the deaminase has no effect on thrombin-induced glycolysis or glycogenolysis (Table 2). Even when the glycolytic pathway is maximally activated because of treatment of the cells with antimycin A (an inhibitor of oxidative phosphorylation), deaminase blocking has no effect on glycolytic rate (Table 2). These observations argue against involvement of AMP-deaminase in a regulatory mechanism of glycolysis (either by ammonia activation of phosphofructokinase or AMP-activation of phosphorylase).

When untreated platelets are incubated with thrombin to induce secretion, the adenylate energy charge remains stable at a level of 0.91 ± 0.02, although up to 30% of the adenine nucleotide is converted to hypoxanthine. In coformycin-treated platelets, an initial drop of the energy charge from 0.91 to 0.85 30 s after stimulation is followed by a return to the control level during the next minute. Since almost no adenylate catabolism takes place in these cells, the energy charge seems to be stabilized by ADP-activation of mitochondrial ATP-synthesis (Ashby et al., 1983).

If hydrogen peroxide is added to a platelet suspension, a strong energy drain occurs, resulting in an irreversible depletion of ATP and accumulation of hypoxanthine. Hydrogen peroxide probably oxidizes intracellular glutathione, which has to be resynthesized at the expense of NADPH. The decrease of NADPH apparently leads to a powerful stimulation of the hexose monophosphate shunt. Under these conditions,
Table 2. Lactate production and glycogenolysis in platelets

<table>
<thead>
<tr>
<th>Condition</th>
<th>Lactate production</th>
<th>Glycogen degradation</th>
<th>Lactate production</th>
<th>Glycogen degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(5 mM extracellular glucose added)</td>
<td>(no extracellular glucose added)</td>
<td>(5 mM extracellular glucose added)</td>
<td>(no extracellular glucose added)</td>
</tr>
<tr>
<td>Control</td>
<td>1.7</td>
<td>&lt;1.0</td>
<td>1.5</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Respiration blocked by antimycin A</td>
<td>12.5</td>
<td>n.d.</td>
<td>12.5</td>
<td>n.d.</td>
</tr>
<tr>
<td>Thrombin-stimulated</td>
<td>6.0</td>
<td>700</td>
<td>6.0</td>
<td>700</td>
</tr>
<tr>
<td>Thrombin+antimycin A</td>
<td>13.7</td>
<td>n.d.</td>
<td>13.4</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Data presented as μmol/min. 10^11 cells (glycogen degradation as glucose units). n.d. = not determined. Data from Ashby et al. (1983).

the adenylate energy charge of untreated cells drops from a level of 0.90 to 0.83 after 10 min. However, in coformycin-treated cells a much larger drop from 0.89 to 0.71 is observed over the same period. Whereas coformycin treatment in the absence of H₂O₂ has no influence on thrombin-induced platelet secretion, a drop of the adenylate energy charge below 0.84 causes significant inhibition of secretion (Ashby et al., 1983). Therefore, platelets treated with H₂O₂ and coformycin show more inhibition of thrombin-induced secretion than platelets treated with H₂O₂ only. The role of platelet AMP-deaminase seems to be stabilization of the adenylate energy charge in the absence of stimulated oxidative phosphorylation or buffering of the energy charge during a temporary imbalance between energy demand and energy production, until mitochondrial ATP-synthesis is sufficiently activated.

V. BRAIN

Neurons produce ammonia under a variety of conditions, such as electrical activity and ischaemia. Ammoniagenesis is raised after the administration of convulsive agents (Folbergrova et al., 1969; Richter & Dawson, 1949), whereas ammonia disappears from the brain during sleep or anaesthesia. Since ammonia does not arise from activity of glutaminase (Weil-Malherbe & Green, 1955; Folbergrova et al., 1969), the glutamate dehydrogenase reaction and the PNC are the major candidates for deamination in the central nervous system.

Schultz & Lowenstein (1976) demonstrated the occurrence of the reactions of the PNC in a gel-filtered, high-speed supernatant of a rat brain homogenate. The reaction was started by addition of IMP, GTP, aspartate and a GTP-regenerating system (phosphoenolpyruvate with endogenous pyruvate kinase) to the extract. Nucleotide interconversions were monitored by repeated spectral scanning in the range of 240–310 nm and by enzymic analysis. Under these conditions, up to 80% of the IMP was converted into ATP. After 75 min the supply of phosphoenolpyruvate became exhausted, leading to a buildup of GDP and inhibition of adenylosuccinate synthetase. ATP was then dephosphorylated by endogenous phosphatases and the resulting AMP was deaminated by adenylate deaminase. Thus, the first turn of the PNC was completed.
A problem in experiments with brain homogenates (as compared to muscle extracts) is the presence of fairly high levels of 5'-nucleotidase (14), adenosine deaminase (15), nucleoside phosphorylase (16) and guanase (17). The following reactions therefore occur in the extract:

\[
\text{AMP (IMP, GMP) + H}_2\text{O} \rightarrow \text{adenosine (inosine, guanosine) + P}_1, \quad (14)
\]

\[
\text{adenosine + H}_2\text{O} \rightarrow \text{inosine + NH}_3, \quad (15)
\]

\[
\text{inosine (guanosine) + P}_1 \rightarrow \text{hypoxanthine (guanine) + ribose-1-P}, \quad (16)
\]

\[
\text{guanine + H}_2\text{O} \rightarrow \text{xanthine + NH}_3. \quad (17)
\]

As long as an excess of the GTP(ATP)-regenerating system is present, hypoxanthine and guanine can be converted back to IMP and GMP, due to the activity of the enzymes hypoxanthine (guanine) phosphoribosyltransferase (18), phosphoribosylpyrophosphate synthetase (19) and phosphoribomutase (20):

\[
\text{hypoxanthine (guanine) + PPriboseP} \rightarrow \text{IMP (GMP) + PP}_1, \quad (18)
\]

\[
\text{ATP + ribose-1-P} \rightarrow \text{PPriboseP + AMP}, \quad (19)
\]

\[
\text{ribose-1-P} \leftrightarrow \text{ribose-5-P}. \quad (20)
\]

Xanthine, however, cannot be converted back to GMP. This makes demonstration of repeated cycling of purine nucleotides more difficult in brain homogenates than it is in muscle homogenates. The presence of adenosine deaminase (15) and guanase (17) in the extract complicates calculations of ammonia stoichiometry, since these reactions form ammonia as well as adenylate deaminase.

The maximum rate of ammonia formation observed in brain after administration of convulsive agents (7.5 μmol/min·g, Folbergrova et al., 1969; Richter & Dawson, 1948) is within the capacity of adenylate deaminase (8.3 μmol/min·g, Schultz & Lowenstein, 1976), but above that of glutamate dehydrogenase (3.5 μmol/min·g in the direction of deamination at its pH-optimum of 8.0 with NAD as cofactor, Schultz & Lowenstein, 1976). Steady-state rates of brain ammoniagenesis (0.037 μmol/min·g in rat brain, 0.13 μmol/min·g in guinea-pig brain, Weil-Malherbe & Green, 1955) compare favourably with the maximum activity of adenylosuccinate synthetase (0.058 μmol/min·g in rat brain, Schultz & Lowenstein, 1976).

Schultz & Lowenstein (1976) therefore proposed that the PNC plays a significant role in ammonia production by nerve cells. It may account for at least one-half of the ammonia production in brain slices in the absence of exogenous substrates.

In a later series of experiments, Schultz & Lowenstein (1978) induced simultaneous firing of as many neurons as possible by electrical shock treatment of rat brain in situ. Subsequently, the brain was removed by freeze-blowing and metabolites were extracted and assayed using standard biochemical techniques.

During 10 s of shock treatment, a large drop in creatine phosphate (to <20 % of the control) and ATP (to 60 % of the control) was accompanied by an increase of ammonia. Ammonia levels continued to rise until after 1 min a plateau of 3 times the control value was reached. The decrease of ATP was accompanied by an increase of ADP, AMP and IMP. Rephosphorylation began immediately after the electrical stimulus was turned off.
as shown by the increase of creatine phosphate. Ammonia formation coincided with the increase in AMP content and the appearance of IMP and adenylosuccinate. As soon as AMP started to decline, ammoniagenesis came to a stop. Ammonia and IMP did not accumulate in stoichiometric amounts, however, ammonia production being much higher than the accumulation of IMP. The discrepancy between the observed IMP and ammonia levels can be explained by removal of IMP via the adenylosuccinate synthetase and 5′-nucleotidase reactions. It is possible, however, that in reality the discrepancy was higher than observed, since part of the ammonia formed may have been removed by the action of glutamate dehydrogenase and glutamine synthetase. During the period of ammonia formation there was no decline of brain glutamate or any increase of the mitochondrial NADH/NAD⁺-ratio, whereas a 2.5-fold decline of α-ketoglutarate occurred.

In summary, Schultz & Lowenstein’s (1978) data show that a large portion of the ammonia produced in the brain during shock treatment is due to the operation of the PNC. A small contribution by the glutamate dehydrogenase reaction cannot be ruled out, however.

VI. VERTEBRATE SKELETAL MUSCLE

Numerous observations suggest that the PNC serves an important function in skeletal muscle:

(a) A unique isozyme of AMP-deaminase is found only in skeletal muscle (Ogasawara et al., 1975, 1978; Solano & Coffee, 1978).

(b) AMP-deaminase activity is an order of magnitude greater in skeletal muscle than in other organs, like brain, liver or kidney. This has been observed in many organisms, including rat (Purzycka, 1962; Lowenstein, 1972; Chandrasena & Hird, 1978), rabbit (Conway & Cooke, 1939), goldfish (Van Waarde, 1981a) and trout (Walton & Cowey, 1977).

(c) Vigorous contraction of fast-twitch glycolytic fibres (but not slow-twitch fibres!) is associated with the accumulation of stoichiometric amounts of IMP and NH₃ (Gerez & Kirsten, 1965; Driedzic & Hochachka, 1976; Goodman & Lowenstein, 1977; Meyer & Terjung, 1979, 1980; Meyer et al., 1980; Van Waarde & Kesbeke, 1983). Ammonia subsequently appears in the efferent venous blood (Wilkerson et al., 1977; Babij et al., 1983; Mutch & Banister, 1983). The activity of muscle AMP-deaminase is under tight control, which appears to be both of a covalent and a non-covalent nature. Nucleotides (ATP, ADP and GTP), inorganic phosphate and inorganic pyrophosphate are effectors (Coffee & Solano, 1977; Ronca-Testoni et al., 1970; Wheeler & Lowenstein, 1979a, b; Van Waarde & Kesbeke, 1981a), whereas a covalent activation mechanism has been described by Rahim et al. (1979).

(d) The muscle isozyme of AMP-deaminase binds to myosin. In rabbit muscle, the binding has been reported to take place mainly at the heavy meromyosin fragment (Ashby & Frieden, 1977, 1978; Barshop & Frieden, 1984; Koretz & Frieden, 1980; Koretz, 1982), but in rat muscle it seems to occur at the light meromyosin fragment (Shiraki et al., 1979a). The association is very tight (Kₐ 60 nm, Shiraki et al., 1979a). Binding leads to a substantial increase of deaminase activity, but actomyosin ATPase is unaffected (Shiraki et al., 1979b). After 30 s stimulation of rat muscle, the ratio of bound to free enzyme is raised fivefold above that in resting muscle. Stimulation also
increases the ammonia content of the muscle to 5 times that in resting state. Thus, a correlation seems to exist between AMP-deaminase binding and muscular ammonia production (Shiraki et al., 1981). Histochemical staining substantiates the association of enzyme activity with the myofibrils (Ashby et al., 1979).

(e) One form of adenylosuccinate synthetase is found predominantly in muscle (Ogawa et al., 1977; Matsuda et al., 1977).

(f) Adenylosuccinate synthetase binds to muscle actin (Ogawa et al., 1978).

(g) Expansion of the pool of citric acid cycle intermediates occurs during muscle contraction, due to aspartate deamination via the adenylosuccinate synthetase and adenylosuccinase reactions (Aragon et al., 1980, 1981). Fumarate thus formed serves as a 'sparker' in the tricarboxylic acid cycle (Scislowski et al., 1982).

(h) Adaptive increases in the level of muscle AMP-deaminase during training have been reported (Hryniewiecki, 1971), although these results could not be confirmed in later investigations (Winder et al., 1974).

(i) Changes occur in the muscle AMP-deaminase isozyme pattern during development. The appearance of the adult isozyme correlates with the onset of movement (Kendrick-Jones & Perry, 1967; Sammons & Chilson, 1978).

More studies have been performed on the operation of the PNC in skeletal muscle than in any other tissue.

One of the ways to assess the function of a metabolic pathway is by the study of mutants, in which a gene coding for an enzyme from the pathway has been deleted. Such a genetic defect does exist in the case of the PNC. A disease of human muscle, myoadenylate deaminase deficiency (MDD) is caused by deletion of the gene that codes for the muscle isozyme of adenylate deaminase (Fishbein et al., 1978, 1979). As a consequence, patients have very low levels of AMP-deaminase in their muscles, whereas other tissues (e.g. erythrocytes, leukocytes, fibroblasts) show normal enzyme activity (Engel et al., 1964; Fishbein et al., 1978, 1980a, b; Shumate et al., 1979, 1980; DiMauro et al., 1980; Heffner, 1980; Scholte et al., 1981; Mercelis et al., 1981; Kar & Pearson, 1981; Hayes et al., 1982; Kelemen et al., 1982). Study of these patients can give interesting clues to the function of the PNC in the contraction process. Generally observed symptoms are weakness (Fishbean et al., 1978; Shumate et al., 1979, 1980; Kar & Pearson, 1981; Mercelis et al., 1981) besides cramping (Fishbean et al., 1978; DiMauro et al., 1980; Kar & Pearson, 1981), stiffness (Hayes et al., 1982) and muscle aches (Shumate et al., 1979; DiMauro et al., 1980; Kar & Pearson, 1981; Hayes et al., 1982; Kelemen et al., 1982) after exercise. Although the existence of a causal relationship between the enzyme defect and the clinical symptoms was initially denied (Shumate et al., 1979, 1980), a relation between the gene deletion and post-exercise myalgia seems to be established (Kelemen et al., 1982). The exact genetic nature of the disease is still unclear, but the gene(s) involved seem to be localized in the autosomes (Kelemen et al., 1982).

In a detailed study of the metabolic consequences of this deficiency, Sabina et al. (1984) made patients and normal subjects perform exercise until exhaustion. They noted the following symptoms:

(i) MDD patients suffer from easy fatigability. They can do only 28% of the
maximal work of their non-MDD counterparts. Both the total work performed and endurance are significantly lower in people suffering from MDD.

(ii) Although the decrease of the level of creatine phosphate during exercise is similar in both groups, the median decrease in total phosphagen per unit work is thus fivefold greater in MDD patients than in the non-MDD group.

(iii) MDD patients suffer from postexertional muscle aches, cramps and pains.

(iv) Lactate production during ischaemia is normal, but there is hardly any IMP accumulation and a strongly depressed ammoniagenesis during ischaemic exercise.

(v) Exercising MDD patients produce much more adenosine than their non-MDD counterparts. In vitro studies of nucleotide catabolism using the adenosine deaminase inhibitor 2'-deoxycoformycin (DCF) suggest that the normal pathway for adenylate breakdown \((\text{AMP} \rightarrow \text{IMP} \rightarrow \text{Ino} \rightarrow \text{Hx})\) is replaced by an alternative route \((\text{AMP} \rightarrow \text{Ado} \rightarrow \text{Ino} \rightarrow \text{Hx})\) in MDD patients. As a consequence, Hx-formation is inhibited by DCF in the patient muscles, whereas in normal subjects, adenylate breakdown is unaffected by DCF.

(vi) In the non-MDD group, a significant decrease of ATP occurs due to conversion of adenylates to IMP. However, in MDD patients there is hardly any decrease of ATP during muscle work.

In theory, the following symptoms could be expected to result from absence of myoadenylate deaminase:

(1) The decrease of ATP-content in skeletal muscle during exercise is normally not associated with an increase in ADP or AMP because AMP-deaminase is activated when the energy charge of the cell falls. Deamination not only prevents buildup of AMP, but also the production of ADP, since the equilibrium of the myokinase reaction is pulled towards ATP formation (see the Introduction). The net effect of the coupled reactions is maximal extraction of energy from the adenylate pool. The adenylate energy charge and ATP phosphorylation potential are kept at the maximum level during a temporary imbalance between energy demand and energy production.

When AMP-deaminase is lacking, IMP-formation cannot occur. ADP and AMP can therefore be expected to increase. Failure to extract all of the energy stored in the adenylate pool might lead to inhibition of actomyosin ATPase and contribute to the more rapid onset of fatigue in the patients (see Sahlin et al., 1978).

This explanation for MDD's symptoms, however, is refuted by the data presented in Table 3. The fall of the energy charge during exhaustive exercise is similar in normal subjects and MDD patients. Neither normal humans nor the patient group show a significant increase of ADP or AMP during muscular work. It is of course possible that due to compartmentation, total pool data do not give adequate information about the actual energy charge and ADP or AMP concentrations close to the actomyosin cross bridges. On the basis of these data, however, it seems unlikely that major differences would exist.

(2) AMP is normally deaminated to IMP during a heavy work load. Since IMP is a charged compound, it cannot pass the plasma membrane or leak out to the circulation. During recovery, IMP is rapidly reaminated and incorporated in the adenine nucleotide pool. In muscles lacking AMP-deaminase, AMP is probably dephosphorylated during exercise. The resulting adenosine leaks out to the circulation, leading to a loss of
Table 3. Changes in the muscle adenylate pool of normal subjects and patients suffering from myoadenylate deaminase deficiency (MDD) during exhaustive exercise (data calculated from Sabina et al., 1984)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal subjects</th>
<th>MDD patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exercise</td>
</tr>
<tr>
<td>ATP</td>
<td>148.7 ± 12.5 (6)</td>
<td>126.8 ± 16.7 (4)</td>
</tr>
<tr>
<td>ADP</td>
<td>20.9 ± 5.5 (8)</td>
<td>20.9 ± 2.4 (4)</td>
</tr>
<tr>
<td>AMP</td>
<td>0.46 ± 0.13 (6)</td>
<td>0.89 (2)</td>
</tr>
<tr>
<td>IMP</td>
<td>0.32 ± 0.19 (6)</td>
<td>0.06 ± 0.01 (3)</td>
</tr>
<tr>
<td>EC</td>
<td>0.93 ± 0.02 (8)</td>
<td>0.93 ± 0.01 (4)</td>
</tr>
<tr>
<td>ATP</td>
<td>107.6 ± 15.0 (6)</td>
<td>117.7 ± 18.1 (4)</td>
</tr>
<tr>
<td>ADP</td>
<td>21.9 ± 5.2 (8)</td>
<td>24.6 ± 2.3 (4)</td>
</tr>
<tr>
<td>AMP</td>
<td>0.55 ± 0.17 (6)</td>
<td>1.09 (2)</td>
</tr>
<tr>
<td>IMP</td>
<td>52.4 ± 14.2 (7)</td>
<td>4.2 ± 1.5 (4)</td>
</tr>
<tr>
<td>EC</td>
<td>0.90 ± 0.02 (8)</td>
<td>0.90 ± 0.01 (4)</td>
</tr>
</tbody>
</table>

Values expressed as means ± standard deviation (nmoles/μmole total creatine). Values for EC, the adenylate energy charge, are without dimension. The only significant difference ($P < 0.05$) is that between the IMP-contents of normal muscles and patient muscles after exercise.

precursors for purine salvage. It might therefore be envisaged that in MDD patients, restoration of the adenylate pool after exercise will be slower than in normal subjects. This explanation for MDD's symptoms is also refuted by Table 3. Both in normal subjects and MDD patients there is no significant decline of the muscle nucleotide pool (ATP + ADP + AMP + IMP) during exercise.

(3) Ammonia has been suggested to play a role in the activation of glycolysis due to its stimulating effect on phosphofructokinase (Abrahams & Younathan, 1971; Lowenstein, 1972; Otto et al., 1976; Sugden & Newsholme, 1975; Tejwani et al., 1973). A significant correlation between AMP-deaminase activity and phosphofructokinase activity exists in different muscle types (Winder et al., 1974). It might therefore be expected that lack of ammonia will lead to a delayed or impaired activation of glycolysis in muscles of MDD patients. This explanation of the clinical symptoms seems to be ruled out by the fact that lactate production during exercise is identical in normal subjects and patients (DiMauro et al., 1980; Merceis et al., 1981; Sabina et al., 1984), although in one individual, a subnormal rise of blood lactate was observed (Hayes et al., 1982). It is possible, however, that a defect of carbohydrate metabolism other than MDD was responsible for impaired glycolysis in this patient (Hayes et al., 1982).

(4) Since AMP is an allosteric activator of phosphorylase b and phosphofructokinase, whereas IMP is much less effective, accumulation of AMP in MDD patients during exercise might lead to excessive glycogenolysis and a delayed 'switching off' of the glycolytic pathway after termination of contraction (see Davuluri et al., 1981). This explanation for MDD's symptoms seems also unlikely, since supranormal lactate formation in MDD patients does not occur (DiMauro et al., 1980; Merceis et al., 1981; Sabina et al., 1984). The increase in AMP concentration during the work load is minor.
The purine nucleotide cycle

(see Table 3) and AMP is probably very rapidly phosphorylated after cessation of exercise.

(5) Ammonia produced in the AMP-deaminase reaction could serve as a buffer for H⁺ arising from ATP-hydrolysis (Hochachka & Mommsen, 1983) or lactate formation (Lowenstein, 1972). Loss of buffering capacity in myoadenylate deficiency might cause inhibition of actomyosin ATP-ase. This explanation of MDD symptoms is unlikely in view of the vast amount of protons produced during exercise which is accompanied by only minor ammoniagenesis. Lactate production in humans exceeds ammonia production 30- to 100-fold (Wilkerson et al., 1977; Fishbein et al., 1978; Babij et al., 1983; Katz et al., 1986). The contribution of ammonia to pH-buffering in muscle seems therefore to be insignificant.

(6) Because lactate production is similar in normal and MDD subjects, whereas the decrease in creatine phosphate per unit work is fivefold greater in the patients, it seems that patient muscles have a lower capacity for aerobic energy production. Evidence that blocking of the PNC gives rise to an impairment of aerobic energy generation also comes from an animal model in which rats were infused with the drug 5-amino-4-imidazolecarboxamide riboside (AICArriboside; Swain et al., 1984; Flanagan et al., 1986).

AICArriboside is not a substrate for nucleoside phosphorylase and therefore does not undergo any detectable phosphorolysis. The compound is phosphorylated to the purine de novo intermediate 5-amino-4-imidazolecarboxamide ribotide (AICAR) by adenosine kinase, AICAR is subsequently formylated to yield IMP (Lowy & Williams, 1977; Zimmerman et al., 1978; Sabina et al., 1982).

When infused in rather low doses, AICArriboside increases the availability of IMP. It can therefore be successfully used to enhance the rate of repletion of the ATP and GTP pools in postischaemic myocardium (Swain et al., 1982). At higher doses, however, AICAR accumulates which is a strong inhibitor of adenylosuccinate lyase (Sabina et al., 1982). Besides AICAR, 5-amino-4-imidazolecarboxamide ribotide triphosphate (ZTP) is formed, since the pyrophosphate group of 5-phosphoribosyl-1-pyrophosphate is transferred to AICAR in a reaction catalysed by 5-phosphoribosyl-1-pyrophosphate synthetase (Zimmerman et al., 1978; Sabina et al., 1983, 1984, 1985).

As a consequence, the aminating steps of the PNC are inhibited.

During both moderate (aerobic) exercise and severe (anaerobic) exercise, animals treated with a high dose of AICArriboside generate the same initial force as control rats. However, a decrease in tension occurs much more rapidly in AICArriboside-treated rats. The total tension developed during the 10 min experimental period is thus much lower in AICArriboside-treated animals than in untreated controls. Anaerobic energy production is unaffected, since lactate formation is identical in both groups. AICArriboside-treatment therefore seems to depress aerobic energy production, which occurs continuously during moderate exercise and initially during severe exercise.

On the basis of metabolite measurements in untreated and AICArriboside-treated animals, Swain et al. (1984) and Flanagan et al. (1986) concluded that AICArriboside prevents the increase in the activities of adenylosuccinate synthetase and adenylosuccinate lyase which normally takes place during exercise. The effects of such a blockade (rapid fatigability, less total tension developed, more phosphagen breakdown per unit workload, normal lactate production) show a striking resemblance to the
clinical symptoms of myoadenylate deaminase deficiency (MDD). The major effect of a disruption of the PNC appears to be impairment of aerobic energy production due to lack of four-carbon intermediates in the TCA cycle.

Essential to this explanation is the concept that the three enzymes of the PNC can be simultaneously active in exercising muscle. Concurrent flux through AMP-deaminase and the adenylosuccinate pathway was implied in the studies of Goodman & Lowenstein (1977) and Lowenstein & Goodman (1978), who noted that exercise results not only in an increase of the level of IMP, but also of that of adenylosuccinate. Simultaneous action of the deaminating and reaminating parts of the cycle was denied by Meyer & Terjung (1980), however. Based on experiments with the adenylosuccinate synthetase inhibitor hadacidin, Meyer & Terjung (1980) and Meyer et al. (1980) concluded that in working red muscle hardly any IMP-reamination occurs, whereas in white muscle reamination is limited to the initial 5 min of a 30 min period of moderate exercise. Meyer & Terjung (1980) therefore suggested that deamination takes place during exercise and reamination occurs almost exclusively during recovery. Although Aragon & Lowenstein (1980) used an identical experimental approach, they reached a different conclusion. Aragon & Lowenstein (1980) followed changes in the levels of tricarboxylic acid cycle intermediates in skeletal muscle of normal and hadacidin-treated rats. The total level of metabolites from the citric acid cycle was found to rise by a factor of two during exercise. Fumarate and malate reached their maximum first, followed by citrate, isocitrate and succinate. These results suggest that fumarate or malate is the endproduct of the anaplerotic reaction occurring under these conditions. Hadacidin had no effect on the levels of Krebs cycle-intermediates in resting muscle, but it caused significant inhibition of the rise in TCA cycle intermediates which occurs during exercise. From simultaneous measurements of nucleotide levels, Aragon & Lowenstein (1980) concluded that the PNC is responsible for at least 72% (and probably more) of the expansion of the citric acid cycle pool during exercise. In contrast to the opinion of Meyer & Terjung (1980), Aragon & Lowenstein (1980) therefore stated that the PNC is a major anaplerotic pathway in exercising rat muscle.

In a very interesting series of experiments, Manfredi & Holmes (1984) incubated gel-filtered protein extracts of rat skeletal muscle with a purine nucleotide pool of physiological composition and size. An ATP-regenerating system or a substrate for energy production were not included. In this in vitro model, the activities of the individual PNC-enzymes and the effect of the composition of the nucleotide pool on these activities could be evaluated. Surprisingly, the activity of each cycle enzyme was observed to increase as the energy charge of the system was lowered over the range 0.99-0.64 (corresponding to a change of the ATP/ADP-ratio of 50 to 1). The activities of the PNC-enzymes increased in parallel with lowering of the charge and the components of the cycle appeared to function as a coordinated unit. Similar results were obtained whether an extract of fast (gastrocnemius) or slow (soleus) muscle was used. The activation of AMP-deaminase at decreasing charge was to be expected, since GTP is an inhibitor whereas ADP is an activator and AMP is the substrate (Ronca-Testoni et al., 1970; Coffee & Solano, 1977; Wheeler & Lowenstein, 1979; Van Waarde & Kesbeke, 1981a), but the activation of adenylosuccinate synthetase was unexpected, since the purified enzyme is inhibited by GDP and ADP (Clark et al., 1977; Faraldo et al., 1983; Matsuda et al., 1977; Muirhead & Bishop, 1974; Ogawa et al., 1977; Van
The purine nucleotide cycle

In the presence of a physiological purine nucleotide pool, however, the availability of IMP seems to be the primary factor determining activity.

Manfredi's and Holmes' experiments demonstrated not only that the aminating and deaminating parts of the cycle can be simultaneously active, but they provided also strong evidence for stabilization of the energy charge by the PNC. Regardless at which value of the energy charge the incubation was started (0.90–0.69), the charge was eventually stabilized at a level of 0.94–0.97. By use of the AMP-deaminase inhibitor coformycin, the authors showed that AMP-deaminase pulled the myokinase equilibrium towards ATP-formation, this mechanism being responsible for stabilization of the energy charge (Manfredi & Holmes, 1984). A function of the PNC in the maintenance of a high ATP/ADP ratio has also been implied by the in vitro studies of Tornheim & Lowenstein (1973, 1974, 1975; reviewed by Tornheim, 1979).

The PNC is not the only reaction which can supply carboxylic acids to the Krebs cycle during exercise. In theory, the following reactions may also be involved.

(i) Alanine aminotransferase (E.C. 2.6.1.2, reaction 21), or the concerted action of alanine aminotransferase and aspartate aminotransferase (E.C. 2.6.1.1, reaction 9):

\[
\text{pyruvate} + \text{glutamate} \rightleftharpoons \text{alpha-ketoglutarate} + \text{alanine} \tag{21} \\
\text{aspartate} + \text{alpha-ketoglutarate} \rightleftharpoons \text{glutamate} + \text{oxaloacetate} \tag{9}
\]

Net: pyruvate + aspartate $\rightleftharpoons$ alanine + oxaloacetate \tag{22}

Reaction (21) produces alpha-ketoglutarate, whereas coupled transamination (22) results in the formation of oxaloacetate.

It is not likely that these reactions would lead to any net increase of the pool of TCA cycle-intermediates during exercise. Meyer & Terjung (1979) noted an increase in pyruvate and alanine and a decrease in glutamate of exercising rat muscle. Since the aminotransferases operate close to equilibrium, these changes would be accompanied by a decrease (and not increase) of alpha-ketoglutarate and oxaloacetate during the period of increased energy demand. In the studies of Goodman & Lowenstein (1977) and Aragon & Lowenstein (1980), aspartate was unchanged whereas pyruvate and alanine were slightly increased and glutamate showed a small decrease. Such changes would be accompanied by a decrease of alpha-ketoglutarate and no change of oxaloacetate if the transaminases maintain equilibrium. The increase of the pool size of TCA-cycle intermediates which occurs during muscular work therefore cannot be due to the action of the aminotransferases.

(ii) Pyruvate carboxylase (E.C. 6.4.1.1, reaction 23):

\[
\text{pyruvate} + \text{ATP} + \text{CO}_2 \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i. \tag{23}
\]

Crabtree et al. (1972) reported that pyruvate carboxylase of vertebrate skeletal muscle is undetectable, whereas other authors (Böttger et al., 1969; Ballard et al., 1970; Van den Thillart & Smit, 1984) measured a small enzyme activity. Spydevold et al. (1976) and Davis et al. (1980) described how intact mitochondria isolated from rat muscle are able to carboxylate pyruvate at a rate of at least 2.5 nmole/mg protein .min at 37° in the
presence of acetyl-coA and malate as a trapping pool. Oxygen uptake by mitochondria resiping on pyruvate is stimulated 3–4 fold by bicarbonate, this stimulation being dependent on ATP. The authors therefore concluded that the pyruvate carboxylase pathway may function in the repletion of citric acid cycle intermediates which is observed when the acetyl coA-concentration is elevated by infusion of fatty acids. It is not clear, however, if pyruvate carboxylase has sufficient activity to contribute significantly to the generation of Krebs-cycle intermediates during exercise. Part of the carboxylase activity in the mitochondrial preparation may also be due to contamination with adipose tissue. Fat cells can be present around skeletal muscle and they possess a high activity of pyruvate carboxylase (Ballard et al., 1970; Aragon & Lowenstein, 1980; Aragon et al., 1981).

(iii) Cytosolic NADP-dependent malic enzyme (E.C. 1.1.1.40, reaction 24):

\[
\text{pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+ \rightarrow \text{malate} + \text{NADP}^+.
\]  

When rat skeletal muscle mitochondria are incubated with pyruvate, bicarbonate and NADPH, cytoplasmatic malic enzyme can replace added malate in stimulating oxidation of acetyl-coA formed from pyruvate by oxidative decarboxylation (Swierczynski, 1980).

(iv) Glutamate dehydrogenase (E.C. 1.4.1.2, reaction 11):

\[
\text{glutamate} + \text{NAD(P)}^+ \rightarrow \text{alpha-ketoglutarate} + \text{NAD(P)H} + \text{NH}_4^+.
\]  

However, activity of glutamate dehydrogenase in the direction of oxidative deamination is virtually undetectable in rat muscle (Aragon & Lowenstein, 1980; Lowenstein, 1972).

Although pyruvate carboxylase and malic enzyme may supply part of the carboxylic acids necessary for the increase of Krebs-cycle flux during exercise, the PNC has a greater activity (Aragon et al., 1980, 1981; Scisowski et al., 1982) and a larger free energy decline (Lehninger, 1970; Lowenstein, 1972). It is therefore not surprising that disruption of the PNC leads to impairment of aerobic energy generation (Flanagan et al., 1986).

Katz et al. (1986) made human subjects perform voluntary leg exercise until fatigue occurred. During contraction, the muscle was kept under ischaemic conditions to avoid loss of ammonia to the circulation and to prevent aerobic phosphocreatine synthesis. The activity of AMP-deaminase was evaluated from the rate of decrease of the adenylate pool size, as determined in biopsy samples (IMP-reamination was negligible under these experimental conditions).

AMP-deaminase activity proved to be related to the fibre composition of the muscle, fast-twitch fibres having the highest amount of enzyme \( (r = 0.92, P < 0.001) \). The activity of the deaminase was not related to fibre lactate content \( (r = 0.27, P > 0.05) \), but positively correlated with the turnover rate of ATP as evaluated from the decline in ATP and PCr and increase in lactate and pyruvate contents \( (r = 0.75, P < 0.001) \). Ammoniagenesis was related to the decline of the size of the adenylate pool \( (r = 0.81, P < 0.001) \).

The authors concluded that:

(a) Muscle acidosis is not essential for the activation of AMP deaminase, since there is no relationship between enzyme activity and lactate content. This conclusion is
further substantiated by the observation that iodo-acetate poisoned rat muscle shows no change in pH during contraction, although the adenylate pool is almost completely converted to IMP (Dudley & Terjung, 1985b). In both fast-twitch red and fast-twitch white fibres, a large increase in lactate content can under certain conditions be accompanied by lack of AMP deamination (Dudley & Terjung, 1985b). Large decreases in pH and activation of AMP-deaminase are therefore to a certain extent independent events.

(b) AMP-deaminase seems to be activated during a temporary imbalance between ATP-consumption and ATP-production, which occurs when a high rate of ATP-turnover is coupled to a low phosphocreatine level. Such a situation will lead to an increase of free AMP and free ADP in the sarcoplasm, with concomitant activation of the enzyme (Katz et al., 1986; see also Dudley & Terjung, 1985b). The greater the capacity of the muscle for aerobic energy production, the less likely is energy shortage and activation of AMP-deaminase to occur (Dudley & Terjung, 1985a). Training of muscles leads to an increased blood supply and increases of mitochondrial content. Trained muscles therefore show a less pronounced decrease of creatine phosphate, less lactate production and a delayed activation of AMP-deaminase during exercise (Dudley & Terjung, 1985a). Elimination of the blood flow on the contrary leads to a more marked decrease of creatine phosphate, elevated levels of lactate and a more rapid activation of AMP-deaminase (Dudley & Terjung, 1985a).

(c) Ammonia does not play an important role in the activation of glycolysis during muscular work. In human muscle, exhaustive exercise is accompanied by accumulation of ammonia to 2.3 μmol/g dry weight. In the same experiment, however, anaerobic glycolysis gave rise to the formation of 75.6 μmol hydrogen ions/g dry weight of muscle. Thus, ammonia can buffer only 3% of the hydrogen ions formed during muscular contraction (Katz et al., 1986). Ammonia activation of phosphofructokinase can be demonstrated under certain in vitro conditions, but ammonia loses its activating effect in the presence of physiological amounts of K⁺ (Sugden & Newsholme, 1975). Detailed observation of the changes in ammonia and lactate contents of muscle during exercise suggest that there is no direct correlation between ammonia level and glycolytic rate. In human muscle, glycolysis is activated almost instantaneously after the onset of contraction, whereas the glycolytic rate remains constant for about 50 s. Yet, during the first 25 s no ammonia accumulation occurs, ammoniagenesis starting only 30 s after the beginning of the exercise (Katz et al., 1986). A similar lack of correlation in time between ammonia production and activation of glycolysis has been observed in rat muscle by Dudley & Terjung (1985a). During recovery of human muscle, ammonia remains elevated for at least four min, although glycolysis is switched off immediately after the termination of exercise (Katz et al., 1986). It is evident that other control mechanisms, like phosphorylation and nucleotide activation of the key enzymes phosphorylase and phosphofructokinase overrule any stimulating effect of ammonia. Under extraordinary conditions (phosphorylase kinase-deficiency), however, AMP-deaminase may be involved in the control of glycolytic rate via IMP-activation of phosphorylase b (Rahim et al., 1976, 1980; Aragon et al., 1980).
Table 4. Distribution of AMP-deaminase in tissues of invertebrates

<table>
<thead>
<tr>
<th>Activity present</th>
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<th>Ref.*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Annelida:</strong></td>
<td></td>
<td>Cephalopoda:</td>
<td></td>
</tr>
<tr>
<td><em>Abarenicola pacifica</em> (gut)</td>
<td>[1]</td>
<td>‘Squid’ (mantle muscle)</td>
<td>[9]</td>
</tr>
<tr>
<td><em>A. vagabunda</em> (gut)</td>
<td>[1]</td>
<td><em>Lozolo pelii</em> (mantle)</td>
<td>[10]</td>
</tr>
<tr>
<td><em>Amphitrite robusta</em> (gut)</td>
<td>[1]</td>
<td><em>Crustacea:</em></td>
<td></td>
</tr>
<tr>
<td><em>Nereis branti</em> (gut)</td>
<td>[1]</td>
<td><em>Insecta:</em></td>
<td></td>
</tr>
<tr>
<td><em>Schizobranchia insignis</em> (gut)</td>
<td>[1]</td>
<td><em>Gastromargus muscus</em> (thorax muscle)</td>
<td>[12]</td>
</tr>
<tr>
<td><strong>Crustacea:</strong></td>
<td></td>
<td><em>Locusta migratoria</em> (leg and thorax muscle)</td>
<td>[12]</td>
</tr>
<tr>
<td>‘Crab’ (claw muscle)</td>
<td>[5]</td>
<td><em>Periplaneta americana</em> (thorax muscle)</td>
<td>[13]</td>
</tr>
<tr>
<td><em>Cancer magister</em></td>
<td>[18]</td>
<td><em>Pelecypoda:</em></td>
<td></td>
</tr>
<tr>
<td><em>Carcinus affinis</em> (claw muscle)</td>
<td>[3]</td>
<td><em>Chlamys opercularis</em> (adductor muscle)</td>
<td>[14]</td>
</tr>
<tr>
<td><em>Chionoecetes opilio</em> (leg muscle)</td>
<td>[6]</td>
<td><em>Crassostrea nippona</em> (midgut gland)</td>
<td>[15]</td>
</tr>
<tr>
<td><em>Homarus americanus</em> (tail muscle)</td>
<td>[7]</td>
<td><em>Pecten yessoensis</em> (adductor muscle)</td>
<td>[16]</td>
</tr>
<tr>
<td><em>Oropectes limosus</em> (tail muscle)</td>
<td>[20]</td>
<td><em>Gastropoda:</em></td>
<td></td>
</tr>
<tr>
<td><em>Paralithodes camtschatica</em></td>
<td>[18]</td>
<td><em>Placopecten magellanica</em> (adductor muscle)</td>
<td>[17]</td>
</tr>
<tr>
<td><em>Rhithropanopeus harrisii</em> (claw)</td>
<td>[3]</td>
<td><em>Platanostea carinun</em> (adductor muscle)</td>
<td>[19]</td>
</tr>
<tr>
<td><strong>Gastropoda:</strong></td>
<td></td>
<td><em>Unio pictorum</em> (adductor)</td>
<td>[3]</td>
</tr>
<tr>
<td><em>Helix aspersa</em> (hepatopancreas)</td>
<td>[8]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>H. pomatia</em> (foot muscle)</td>
<td>[3]</td>
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<td><strong>Other mollusca:</strong></td>
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</tr>
<tr>
<td><em>Haliotis</em> sp.</td>
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</table>


Table 5. Changes in muscle adenine nucleotide levels during exercise

(All values in μmol/g wet weight, except those indicated by *, which are in μmol/g dry weight. AEC = Adenylate energy charge (value without dimension); TAN = total level of adenine nucleotides. The upper line for a particular species indicates the control condition; the lower line is the exercised condition. Significant differences between control and exercised condition are indicated by asterisks (*).)

<table>
<thead>
<tr>
<th>Species</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN</th>
<th>AEC</th>
<th>Ref.*</th>
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<tr>
<td>Control</td>
<td>3.56</td>
<td>1.96</td>
<td>0.52</td>
<td>5.14</td>
<td>0.80</td>
<td>[1]</td>
</tr>
<tr>
<td>12 h exercise</td>
<td>2.52*</td>
<td>1.94*</td>
<td>0.70</td>
<td>5.16</td>
<td>0.68*</td>
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<tr>
<td>Control</td>
<td>10.29</td>
<td>3.17</td>
<td>0.71</td>
<td>14.17</td>
<td>0.84</td>
<td>[2]*</td>
</tr>
<tr>
<td>2 h exercise</td>
<td>8.41*</td>
<td>4.08*</td>
<td>1.65*</td>
<td>14.05</td>
<td>0.74*</td>
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</table>
### The purine nucleotide cycle

**Table 5 (cont.)**

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<thead>
<tr>
<th>Species</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN</th>
<th>AEC</th>
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<tr>
<td>Control</td>
<td>5'25</td>
<td>0'66</td>
<td>0'06</td>
<td>5'97</td>
<td>0'93</td>
<td>[3]</td>
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<tr>
<td>Exhausted</td>
<td>0'83*</td>
<td>1'89*</td>
<td>2'74*</td>
<td>5'46</td>
<td>0'33*</td>
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<tr>
<td>Control</td>
<td>8'81</td>
<td>1'38</td>
<td>0'16</td>
<td>10'35</td>
<td>0'92</td>
<td>[4]</td>
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<tr>
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<td>8'82</td>
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<td>5'3</td>
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<td>Control</td>
<td>7'43</td>
<td>0'94</td>
<td>0'08</td>
<td>8'45</td>
<td>0'93</td>
<td>[6]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>4'47*</td>
<td>2'76*</td>
<td>1'42*</td>
<td>8'65</td>
<td>0'67*</td>
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<td>Control</td>
<td>20'4</td>
<td>2'9</td>
<td>2'1</td>
<td>25'4</td>
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<tr>
<td>Exhausted</td>
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<td>7'6*</td>
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<td>3 m flight</td>
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<td>0'43</td>
<td>0'06</td>
<td>5'55</td>
<td>0'95</td>
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</tr>
<tr>
<td>Control</td>
<td>4'32*</td>
<td>1'10*</td>
<td>0'12*</td>
<td>5'54</td>
<td>0'88*</td>
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<tr>
<td>Jumping exercise</td>
<td>6'02</td>
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<td>6'85</td>
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</tr>
<tr>
<td></td>
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<td>1'96*</td>
<td>0'92*</td>
<td>8'48</td>
<td>0'78*</td>
<td></td>
</tr>
<tr>
<td><strong>Teleostei</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Carassius auratus</em></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
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<td>0'51</td>
<td>0'02</td>
<td>3'56</td>
<td>0'92</td>
<td>[10]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>1'97*</td>
<td>0'73</td>
<td>0'10*</td>
<td>2'79</td>
<td>0'81*</td>
<td></td>
</tr>
<tr>
<td><em>Cyprinus carpio</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>4'12</td>
<td>0'97</td>
<td>0'07</td>
<td>5'16</td>
<td>0'89</td>
<td>[11]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>1'87*</td>
<td>0'73*</td>
<td>0'08*</td>
<td>2'68*</td>
<td>0'83</td>
<td></td>
</tr>
<tr>
<td><em>Macrozoarces americanus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2'53</td>
<td>0'29</td>
<td>0'08</td>
<td>2'90</td>
<td>0'92</td>
<td>[12]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>1'77*</td>
<td>0'49</td>
<td>0'26*</td>
<td>2'52</td>
<td>0'86*</td>
<td></td>
</tr>
<tr>
<td><strong>Mammalia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>24'3</td>
<td>3'0</td>
<td>0'2</td>
<td>27'5</td>
<td>0'94</td>
<td>[13]*</td>
</tr>
<tr>
<td>Exhausted</td>
<td>19'6*</td>
<td>3'8*</td>
<td>0'2</td>
<td>23'6*</td>
<td>0'91*</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5'17</td>
<td>0'85</td>
<td>0'12</td>
<td>6'14</td>
<td>0'91</td>
<td>[14]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>2'91*</td>
<td>1'29*</td>
<td>0'15</td>
<td>4'35*</td>
<td>0'82*</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6'99</td>
<td>0'93</td>
<td>0'05</td>
<td>7'64</td>
<td>0'97</td>
<td>[15]</td>
</tr>
<tr>
<td>Exhausted</td>
<td>5'14*</td>
<td>0'72*</td>
<td>0'03*</td>
<td>5'89*</td>
<td>0'93*</td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>26'6</td>
<td>3'7</td>
<td>0'4</td>
<td>30'7</td>
<td>0'93</td>
<td>[16]*</td>
</tr>
<tr>
<td>Exhausted</td>
<td>16'9*</td>
<td>3'5</td>
<td>0'5*</td>
<td>20'9*</td>
<td>0'86*</td>
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</tr>
</tbody>
</table>

### Table 6. Changes in the adenylate pool of muscle during severe exercise

(Calculated from the data presented in Table 5.)

<table>
<thead>
<tr>
<th>Species</th>
<th>$\Delta$ATP (%)</th>
<th>$\Delta$TAN (%)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Invertebrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arenicola marina</td>
<td>$-29.2$</td>
<td>$+0.4$</td>
<td></td>
</tr>
<tr>
<td>Tubifex sp.</td>
<td>$-18.3$</td>
<td>$+0.1$</td>
<td>$183$</td>
</tr>
<tr>
<td>Chlamys opercularis</td>
<td>$-84.2$</td>
<td>$-8.5$</td>
<td>$10$</td>
</tr>
<tr>
<td>Placopecten magellanicus</td>
<td>$-35.9$</td>
<td>$-14.8$</td>
<td>$25$</td>
</tr>
<tr>
<td>Loligo pealii</td>
<td>$-60.4$</td>
<td>$-3.2$</td>
<td>$19$</td>
</tr>
<tr>
<td>Cherax destructor</td>
<td>$-39.8$</td>
<td>$+2.1$</td>
<td></td>
</tr>
<tr>
<td>Crangon crangon</td>
<td>$-45.6$</td>
<td>$+10.6$</td>
<td></td>
</tr>
<tr>
<td>Schistocerca gregaria</td>
<td>$-14.6$</td>
<td>$-0.2$</td>
<td>$73$</td>
</tr>
<tr>
<td>Locusta migratoria</td>
<td>$-7.0$</td>
<td>$+23.8$</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>$-37$</td>
<td>$+1$</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>$24$</td>
<td>$11$</td>
<td></td>
</tr>
<tr>
<td><strong>Vertebrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carassius auratus</td>
<td>$-36.9$</td>
<td>$-21.6$</td>
<td>$1.7$</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>$-54.6$</td>
<td>$-48.1$</td>
<td>$1.1$</td>
</tr>
<tr>
<td>Macroaoarces americanus</td>
<td>$-30.0$</td>
<td>$-13.1$</td>
<td>$2.3$</td>
</tr>
<tr>
<td>Human</td>
<td>$-19.3$</td>
<td>$-14.2$</td>
<td>$1.4$</td>
</tr>
<tr>
<td>Human</td>
<td>$-49.0$</td>
<td>$-20.2$</td>
<td>$1.7$</td>
</tr>
<tr>
<td>Mouse</td>
<td>$-26.5$</td>
<td>$-22.9$</td>
<td>$1.2$</td>
</tr>
<tr>
<td>Rat</td>
<td>$-36.5$</td>
<td>$-31.9$</td>
<td>$1.1$</td>
</tr>
<tr>
<td>Mean</td>
<td>$-36$</td>
<td>$-26$</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>$12$</td>
<td>$12$</td>
<td></td>
</tr>
<tr>
<td><strong>Difference between vertebrates and invertebrates</strong></td>
<td>Not significant</td>
<td>$P &lt; 0.5%$</td>
<td></td>
</tr>
</tbody>
</table>

(Student’s $t$ test)

### VII. INVERTEBRATE TISSUES

In invertebrate tissues, the activity of AMP-deaminase is generally much lower than in the corresponding organs of vertebrates. When low substrate concentrations ($0.15$ mM) are used in a spectrophotometric assay, the muscle enzyme is usually undetectable (Umiastowski, 1964). At saturating AMP-levels, however, enzyme activity can be measured in some species (Umiastowski, 1964). In other species, especially insects and bivalves, the enzyme seems to be really lacking (see Table 4).

Many authors have described the breakdown of purine nucleotides occurring in dead animals upon storage at $0^\circ$C or at freezing temperatures. Purine catabolism is of commercial interest, since one of the metabolites, hypoxanthine, has an unpleasant flavour and is thus spoiling the taste of fishery products. A breakdown pattern in which ADP, AMP, IMP, inosine and hypoxanthine show subsequent accumulation is strong evidence for the presence of AMP-deaminase in the muscle. Such evidence has been presented for certain Crustacea (Dingle & Hines, 1974; Fraser Hiltz & Bishop, 1975; Groninger & Brandt, 1969, 1970; see Table 4). In other invertebrates, like squid (Saito et al., 1958) and scallop (Fraser Hiltz & Dyer, 1970) purine degradation follows the route:

$$\text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{adenosine} \rightarrow \text{inosine} \rightarrow \text{hypoxanthine}.$$
### The purine nucleotide cycle

#### Table 7. Changes of adenine nucleotide levels in tissues during anoxia

(Upper line for a particular species is the control condition, lower line is the anoxic condition. Presentation of data similar to Table 5.)

<table>
<thead>
<tr>
<th>Organism</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>TAN</th>
<th>AEC</th>
<th>Ref.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coelenterata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bunodosoma cavernata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.62</td>
<td>0.28</td>
<td>0.07</td>
<td>0.97</td>
<td>0.78</td>
<td>[1]</td>
</tr>
<tr>
<td>3 h anoxia</td>
<td>0.33*</td>
<td>0.42*</td>
<td>0.24*</td>
<td>0.99</td>
<td>0.54*</td>
<td></td>
</tr>
<tr>
<td>Annelida</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arenicola marina</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.73</td>
<td>0.85</td>
<td>0.30</td>
<td>4.88</td>
<td>0.85</td>
<td>[2]</td>
</tr>
<tr>
<td>12 h anoxia</td>
<td>2.92*</td>
<td>1.16*</td>
<td>0.66*</td>
<td>4.74</td>
<td>0.74*</td>
<td></td>
</tr>
<tr>
<td>Tubific sp.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10.29</td>
<td>3.17</td>
<td>0.71</td>
<td>14.17</td>
<td>0.84</td>
<td>[3]</td>
</tr>
<tr>
<td>2 h anoxia</td>
<td>8.41*</td>
<td>4.08*</td>
<td>1.65*</td>
<td>14.05</td>
<td>0.74*</td>
<td></td>
</tr>
<tr>
<td>Pelecypoda</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.15</td>
<td>0.69</td>
<td>0.11</td>
<td>2.95</td>
<td>0.85</td>
<td>[4]</td>
</tr>
<tr>
<td>5 d anoxia</td>
<td>1.30*</td>
<td>1.34*</td>
<td>0.52*</td>
<td>3.16</td>
<td>0.62*</td>
<td></td>
</tr>
<tr>
<td>Crustacea</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Orconectes limosus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.09</td>
<td>0.41</td>
<td>0.06</td>
<td>4.56</td>
<td>0.94</td>
<td>[5]</td>
</tr>
<tr>
<td>16 h anoxia</td>
<td>3.75</td>
<td>1.12*</td>
<td>0.36*</td>
<td>5.23</td>
<td>0.82*</td>
<td></td>
</tr>
<tr>
<td>Teleostei</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anguilla anguilla</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.79</td>
<td>0.33</td>
<td>0.05</td>
<td>2.16</td>
<td>0.90</td>
<td>[6]</td>
</tr>
<tr>
<td>6 h anoxia</td>
<td>1.14</td>
<td>0.34</td>
<td>0.16*</td>
<td>1.63</td>
<td>0.71*</td>
<td></td>
</tr>
<tr>
<td><em>Carassius auratus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.22</td>
<td>0.72</td>
<td>0.12</td>
<td>4.06</td>
<td>0.88</td>
<td>[7]</td>
</tr>
<tr>
<td>Red muscle 12 h anoxia</td>
<td>2.44</td>
<td>0.74</td>
<td>0.18</td>
<td>3.36</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td><em>Carassius auratus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.46</td>
<td>0.81</td>
<td>0.05</td>
<td>6.32</td>
<td>0.93</td>
<td>[7]</td>
</tr>
<tr>
<td>White muscle 12 h anoxia</td>
<td>4.17</td>
<td>0.82</td>
<td>0.10*</td>
<td>5.09</td>
<td>0.89*</td>
<td></td>
</tr>
<tr>
<td><em>Platichthys flesus</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.63</td>
<td>0.77</td>
<td>0.13</td>
<td>5.53</td>
<td>0.91</td>
<td>[8]</td>
</tr>
<tr>
<td>29 h hypoxia</td>
<td>3.79*</td>
<td>0.83</td>
<td>0.18</td>
<td>4.80</td>
<td>0.86*</td>
<td></td>
</tr>
</tbody>
</table>


This suggests AMP-deaminase activity in the latter species is much lower than the activity of 5'-nucleotidase.

The properties of AMP-deaminase in invertebrate muscle (when the enzyme is present) seem different from those in muscle of vertebrates. The invertebrate enzyme generally has a much lower affinity for its substrate and a much lower maximal velocity than its vertebrate counterpart (Bishop & Barnes, 1971; Gibbs & Bishop, 1977; MacDonnell & Tillinghast, 1973; Stankiewicz, 1982). As a consequence, the behaviour of the adenylate pool during an imbalance between energy consumption and energy production is different from that in vertebrates.

Changes in the levels of purine nucleotides during severe exercise have been reviewed in the Tables 5 and 6. It is clear that in both vertebrates and invertebrates, exercise results in a significant drop of the ATP-content of muscle. In vertebrates, this is
Table 8. Changes of purine nucleotides in muscle during anoxia
(Calculated from the data presented in Table 7.)

<table>
<thead>
<tr>
<th>Species</th>
<th>ATP (%)</th>
<th>TAN (%)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invertebrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bunodosoma cavernata</em></td>
<td>-46.8</td>
<td>+2.1</td>
<td>—</td>
</tr>
<tr>
<td><em>Arenicola marina</em></td>
<td>-21.7</td>
<td>-2.9</td>
<td>7.5</td>
</tr>
<tr>
<td><em>Tubifex</em> sp.</td>
<td>-18.3</td>
<td>-0.1</td>
<td>18.3</td>
</tr>
<tr>
<td><em>Mytilus edulis</em></td>
<td>-39.5</td>
<td>+7.1</td>
<td>—</td>
</tr>
<tr>
<td><em>Orconectes limosus</em></td>
<td>-8.3</td>
<td>+14.7</td>
<td>—</td>
</tr>
<tr>
<td>Mean</td>
<td>-27</td>
<td>+4</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>16</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Vertebrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anguilla anguilla</em></td>
<td>-36.3</td>
<td>-24.5</td>
<td>1.5</td>
</tr>
<tr>
<td><em>Carassius auratus</em> (red)</td>
<td>-24.2</td>
<td>-17.2</td>
<td>1.4</td>
</tr>
<tr>
<td><em>Carassius auratus</em> (white)</td>
<td>-23.6</td>
<td>-19.5</td>
<td>1.2</td>
</tr>
<tr>
<td><em>Platichthys flesus</em></td>
<td>-18.1</td>
<td>-13.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Mean</td>
<td>-26</td>
<td>-19</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>8</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Difference between</td>
<td>Not significant</td>
<td>$P &lt; 0.5%$</td>
<td></td>
</tr>
<tr>
<td>vertebrates and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>invertebrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Student's t test)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

accompanied by activation of AMP-deaminase and a decline of the total adenine nucleotide pool. No such activation occurs in invertebrates, however, the decline of the nucleotide pool being small or even absent. As a consequence, the adenylate energy charge shows a much greater drop in invertebrate muscle during exercise.

Another metabolic situation in which imbalance between ATP-consumption and ATP-production is likely to occur is environmental anoxia. Purine metabolism in anoxic muscle has been reviewed in the Tables 7 and 8. A similar pattern is observed as during exercise. In both vertebrates and invertebrates, anoxia results in a significant drop of muscle ATP-content. In vertebrates, AMP-deaminase is activated and purine nucleotides are broken down to IMP (values not shown in Table 7), but in invertebrates, total adenine nucleotide content does not show any decline.

We conclude that:

(a) muscles of many invertebrate species lack adenylate deaminase, the deaminating enzyme from the purine nucleotide cycle;

(b) even when adenylate deaminase is present, the enzyme seems not to be activated during an imbalance between energy production and energy consumption;

(c) the PNC therefore does not operate in the same way as in vertebrate muscle.

VIII. CONCLUSION

The absence of adenylate deaminase from certain muscles indicates that deamination of AMP is not essential to the contraction process. In the absence of the enzyme, the functions of the purine nucleotide cycle seem to be performed by other metabolic pathway(s).

It is interesting to note that insect flight muscle, an aerobic tissue with an enormous
The purine nucleotide cycle

metabolic rate (Beenackers et al., 1984), lacks adenylate deaminase and therefore does not possess a complete purine nucleotide cycle. In this muscle, dicarboxylic acids are supplied to the Krebs cycle by the action of the enzyme pyruvate carboxylase. Insect muscle contains an enormous amount of this enzyme in comparison to vertebrate muscle (Crabtree et al., 1972; Lowenstein, 1972). Thus, the anaplerotic function of the PNC seems to be taken over by pyruvate carboxylase. It is not known whether other invertebrate muscles in which the PNC is lacking have a similar replacement. Adductor muscle of bivalves shows rather high levels of phosphoenolpyruvate carboxykinase, which may also supply intermediates to the tricarboxylic acid cycle (Crabtree et al., 1972).

Stabilization of the adenylate ‘energy charge’ (or phosphorylation potential) is an established function of the PNC in the cytosol of vertebrate cells, but such stabilization seems to be lacking in invertebrates. Some metabolic parameters (like the rate of protein synthesis, Edwards et al., 1979) are strongly dependent on the energy charge, whereas others (like the synthesis of lipids, Farber, 1973) show little variation over a broad range of charge. Since the charge is strongly stabilized in vertebrates, but much less in invertebrates, it seems reasonable to assume that some energy-consuming process is not allowed to slow down in vertebrates whereas it can drop in invertebrates. It is tempting to speculate that this process might be the action of ion pumps in cellular membranes. Invertebrate membranes generally have a much lower density of ion channels and also show the possibility of channel arrest (Hochachka & Guppy, 1987).

IX. SUMMARY

1. The operation of the purine nucleotide cycle, consisting of the enzymes adenylate deaminase (E.C. 3.5.4.6), adenylosuccinate synthetase (E.C. 6.3.4.4) and adenylosuccinate lyase (E.C. 4.3.2.2), has been reviewed with reference to its metabolic function in animal tissues.

2. Abundant evidence, both from in vitro and in vivo studies, suggests that the purine nucleotide cycle serves to stabilize the adenylate ‘energy charge’ (or ‘phosphorylation potential’) in the cytoplasm of vertebrate cells during a temporary imbalance between ATP-consumption and ATP-production. This stabilization, however, is absent or much less efficient in tissues of invertebrates.

3. The hypothesis that AMP-deaminase is involved in the regulation of glycolysis is not supported by recent work. In a variety of cell types, including skeletal muscle and blood platelets, blocking of AMP-deaminase activity (due to a genetic defect or to pharmacological inhibition) is without effect on the glycolytic rate. Detailed kinetic and histochemical analysis of energy metabolism shows lack of correlation between AMP-deaminase activity and glycolysis in skeletal muscle during exercise.

4. The purine nucleotide cycle appears to control the level of citric acid cycle intermediates in skeletal muscle. Pharmacological inhibition of adenylosuccinate lyase or adenylosuccinate synthetase leads to a reduced availability of four-carbon ‘sparker’ molecules to the Krebs cycle with a concomitant impairment of aerobic energy production during muscular work.

5. The cycle appears to be a major pathway for amino acid deamination in skeletal muscle and brain of vertebrates, but not in kidney or liver.
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XII. ADDENDUM

Since the original submission of this manuscript, two papers have been published which deal with the difference in adenylate catabolism between vertebrates and invertebrates. Fijisawa & Yoshino (1987) examined the activities of AMP-deaminase,
5'-nucleotidase and adenosine deaminase in muscles from vertebrates and invertebrates at saturating substrate concentrations. They confirmed the result quoted in Table 4, indicating that AMP-deaminase is undetectable in muscle of *Haliotis gigantea*. The enzyme is also lacking in the cuttlefish *Sepia subculeata*, but present in low amounts in muscle from the Crustacea *Pandalus borealis*, *Paracaus japonicus* and *Neptunus trituberculosi*, the Mollusca *Scapharca broughtoni*, *Rapona thomasiana*, *Tegillarca granosa*, *Batillus cornulus*, *Ampullaria* sp. and *Schizithaerus keene*, and the Cephalopoda *Doryteuthis kensaki*, *Todarodus pacificus*, *Watasenia scintillans* and *Doryteuthis bleeker*. Raffin & Thebault (1987) purified an AMP-deaminase from the tail muscle of the Crustacean *Palaemon serratus*. The enzyme was shown to be very different from that in vertebrate muscle; both its specific activity and its affinity for AMP were very low. Thus, the essential difference between vertebrates and invertebrates in regards to the regulation of the PNC has been observed by many authors.